

The following is a complete listing of all claims in the application, with an indication of the status of each:

Listing of claims:

Claims 1-111. Cancelled.

112. (Previously presented). An isolated or purified cell which is recombinant or genetically modified to contain and co-express

i) a cytidine monophosphate sialic acid (CMP- SA)-synthase gene wherein said CMP- SA synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said polypeptide is

a) a polypeptide represented by SEQ ID NO:4 or

b) a variant polypeptide thereof that contains conservative amino acid substitutions and in which CMP-SA-synthase structure and function are conserved, and

ii) a sialic acid phosphate synthase (SAS) gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said polypeptide is

a) a polypeptide represented by SEQ ID NO: 6 or a variant polypeptide thereof that contains conservative amino acid substitutions and in which SAS structure and function are conserved, or

b) a polypeptide represented by SEQ ID NO: 8 or a variant polypeptide thereof that contains conservative amino acid substitutions and in which SAS structure and function are conserved, and

wherein said cell is capable of producing donor substrate CMP-SA above levels produced before said cell was made recombinant or genetically modified, when provided ManNAc.

113. (Previously presented) The isolated or purified cell of claim 112, which is an insect cell.

114. (Previously presented) The insect cell of claim 113, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

115. (Previously presented) The isolated or purified cell of claim 112, which is a yeast cell.

116. (Previously presented) The isolated or purified cell of claim 112, which is a plant cell.

117. (Previously presented) The isolated or purified cell of claim 112, which is a bacterial cell.

118. (Previously presented) The isolated or purified cell of claim 112, which is a fungal cell.

119. (Previously presented) The isolated or purified cell of claim 112, wherein said cell is a mammalian cell.

120. (Previously presented) The isolated or purified cell of claim 112, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

121. (Previously presented) The isolated or purified cell of claim 112 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

122. (Previously presented) The isolated or purified cell of claim 112 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

123. (Previously presented) The isolated or purified cell of claim 122, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

124. (Previously presented) The isolated or purified cell of claim 123, wherein said epimerase is UDP-GlcNAc-2 epimerase.

125. (Previously presented) The isolated or purified cell of claim 123, wherein said epimerase is GlcNAc-2 epimerase.

126. (Previously presented) The isolated or purified cell of claim 112, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

127. (Previously presented). An isolated or purified cell which is recombinant or genetically modified to contain or co-express

i) a cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP-SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase (SAS) gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said SAS gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

and wherein said cell is capable of producing donor substrate CMP-SA above levels produced before said cell was made recombinant or genetically modified, when provided with ManNAc.

128. (Previously presented) The isolated or purified cell of claim 127, which is an insect cell.

129. (Previously presented) The insect cell of claim 128, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

130. (Previously presented) The isolated or purified cell of claim 127, which is a yeast cell.

131. (Previously presented) The isolated or purified cell of claim 127, which is a plant cell.

132. (Previously presented) The isolated or purified cell of claim 127, which is a bacterial cell.

133. (Previously presented) The isolated or purified cell of claim 127, which is a fungal cell.

134. (Previously presented) The isolated or purified cell of claim 127, wherein said cell is a mammalian cell.

135. (Previously presented) The isolated or purified cell of claim 127, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

136. (Previously presented) The isolated or purified cell of claim 127 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

137. (Previously presented) The isolated or purified cell of claim 127 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

138. (Previously presented) The isolated or purified cell of claim 137, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

139. (Previously presented) The isolated or purified cell of claim 138, wherein said epimerase is UDP-GlcNAc-2 epimerase.

140. (Previously presented) The isolated or purified cell of claim 138, wherein said epimerase is GlcNAc-2 epimerase.

141. (Previously presented) The isolated or purified cell of claim 127, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

142-150. (Cancelled)

151. (Previously presented) An isolated or purified cell that is recombinant or genetically modified to contain and co-express

i) a cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said polypeptide is

a) a polypeptide represented by SEQ ID NO:4 or

b) a variant of the polypeptide represented by SEQ ID NO:4 that contains conservative amino acid substitutions and in which CMP-SA structure and function are conserved; and

ii) a sialic acid phosphate synthase (SAS) gene, wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said polypeptide is

a) a polypeptide represented by SEQ ID NO: 6; or

b) a variant of the polypeptide represented by SEQ ID NO:6 that contains conservative amino acid substitutions and in which SAS structure and function are conserved; or

c) a polypeptide represented by SEQ ID NO: 8; or

d) a variant of the polypeptide represented by SEQ ID NO: 8 that contains conservative amino acid substitutions and in which SAS structure and function are conserved,

said cell producing the donor substrate CMP-SA above a level produced before said cell was made recombinant or genetically modified, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonoic acid).

152. (Previously presented) The isolated or purified cell of claim 151, which is an insect cell.

153. (Previously presented) The insect cell of claim 152, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmene acrea*; and,
- (d) *Drosophila*.

154. (Previously presented) The isolated or purified cell of claim 151, which is a yeast cell.

155. (Previously presented) The isolated or purified cell of claim 151, which is a plant cell.

156. (Previously presented) The isolated or purified cell of claim 151, which is a bacterial cell.

157. (Previously presented) The isolated or purified cell of claim 151, which is a fungal cell.

158. (Previously presented) The isolated or purified cell of claim 151, which is a mammalian cell.

159. (Previously presented) The isolated or purified cell of claim 151, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

160. (Previously presented) An isolated or purified cell that is recombinant or genetically modified to contain and co-express

i) a cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP-SA synthase gene comprises

- a) a nucleotide sequence represented by SEQ ID NO: 3, or
- b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said SAS gene comprises

- a) a nucleotide sequence represented by SEQ ID NO: 5, or
- b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

said cell producing the donor substrate CMP-SA above a level produced before said cell was made recombinant or genetically modified, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-glycero-D-galacto-nonoic acid).

161. (Previously presented) The isolated or purified cell of claim 160, which is an insect cell.

162. (Previously presented) The insect cell of claim 161, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,

(d) *Drosophila*.

163. (Previously presented) The isolated or purified cell of claim 160, which is a yeast cell.

164. (Previously presented) The isolated or purified cell of claim 160, which is a plant cell.

165. (Previously presented) The isolated or purified cell of claim 160, which is a bacterial cell.

166. (Previously presented) The isolated or purified cell of claim 160, which is a fungal cell.

167. (Previously presented) The isolated or purified cell of claim 160, which is a mammalian cell.

168. (Previously presented) The isolated or purified cell of claim 160, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

169-184. (Cancelled)

185. (Previously presented) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said polypeptide is

a) a polypeptide represented by SEQ ID NO:4 or

b) a variant of the polypeptide represented by SEQ ID NO:4 that contains conservative amino acid substitutions and in which CMP-SA structure and function are conserved; and

ii) a sialic acid phosphate synthase (SAS) gene, wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said polypeptide is

- a) a polypeptide represented by SEQ ID NO: 6; or
- b) a variant of the polypeptide represented by SEQ ID NO: 6 that contains conservative amino acid substitutions and in which SAS structure and function are conserved; or
- c) a polypeptide represented by SEQ ID NO: 8; or
- d) a variant of the polypeptide represented by SEQ ID NO: 8 that contains conservative amino acid substitutions and in which SAS structure and function are conserved,

said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, when provided with ManNAc.

186. (Previously presented) The isolated or purified cell of claim 185, which is an insect cell.

187. (Previously presented) The insect cell of claim 186, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

188. (Previously presented) The isolated or purified cell of claim 185, which is a yeast cell.

189. (Previously presented) The isolated or purified cell of claim 185, which is a plant cell.

190. (Previously presented) The isolated or purified cell of claim 185, which is a bacterial cell.

191. (Previously presented) The isolated or purified cell of claim 185, which is a fungal cell.

192. (Previously presented) The isolated or purified cell of claim 185, which is a mammalian cell.

193. (Previously presented) The isolated or purified cell of claim 185, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

194. (Previously presented) The isolated or purified cell of claim 185 wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

195. (Previously presented) The cell of claim 185 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

196. (Previously presented) The cell of claim 185 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

197. (Previously presented) The cell of claim 196, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

198. (Previously presented) The cell of claim 197, wherein said epimerase is UDP-GlcNAc-2 epimerase.

199. (Previously presented) The cell of claim 197, wherein said epimerase is GlcNAc-2 epimerase.

200. (Previously presented) The cell of claim 185 wherein said cell also contains SA precursor GlcNAc above endogenous levels as a result of supplementation of cell growth medium with SA precursor GlcNAc.

201. (Previously presented) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a cytidine monophosphate -sialic acid (CMP-SA) - synthase gene, wherein said CMP-SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP-SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said sialic acid phosphate synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, when provided with ManNAc.

202. (Previously presented) The isolated or purified cell of claim 201, which is an insect cell.

203. (Previously presented) The insect cell of claim 202, wherein said insect cell is of a species selected from the group consisting of:

(a) *Spodoptera frugiperda*;

(b) *Trichoplusia ni*;

(c) *Estigmena acrea*; and,

(d) *Drosophila*.

204. (Previously presented) The isolated or purified cell of claim 201, which is a yeast cell.

205. (Previously presented) The isolated or purified cell of claim 201, which is a plant cell.

206. (Previously presented) The isolated or purified cell of claim 201, which is a bacterial cell.

207. (Previously presented) The isolated or purified cell of claim 201, which is a fungal cell.

208. (Previously presented) The isolated or purified cell of claim 201, which is a mammalian cell.

209. (Previously presented) The isolated or purified cell of claim 201, wherein the donor substrate CMP-SA is CMP-Neu5Ac (cytidine monophosphate-*N*-acetylneuraminic acid).

210. (Previously presented) The isolated or purified cell of claim 201 wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

211. (Previously presented) The cell of claim 201 wherein said cell is provided with ManNAc by addition of ManNAc to media in which said cell is grown.

212. (Previously presented) The cell of claim 201 wherein said cell is provided with ManNAc by genetically engineering said cell to catalyze synthesis of ManNAc.

213. (Previously presented) The cell of claim 212, wherein said cell is genetically engineered to contain or express an epimerase that synthesizes ManNAc.

214. (Previously presented) The cell of claim 213, wherein said epimerase is UDP-GlcNAc-2 epimerase.

215. (Previously presented) The cell of claim 213, wherein said epimerase is GlcNAc-2 epimerase.

216. (Previously presented) The cell of claim 201 wherein said cell also contains SA precursor GlcNAc above endogenous levels as a result of supplementation of cell growth medium with SA precursor GlcNAc.

217- 225. (Cancelled)

226. (Previously presented) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP- SA-synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said polypeptide is

- a) a polypeptide represented by SEQ ID NO:4 or
- b) a variant of the polypeptide represented by SEQ ID NO:4 that contains conservative amino acid substitutions and in which CMP-SA synthase structure and function are conserved; and

ii) a sialic acid phosphate synthase (SAS) gene, wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein polypeptide is

- a) a polypeptide represented by SEQ ID NO: 6; or
- b) a variant of the polypeptide represented by SEQ ID NO:6 that contains conservative amino acid substitutions and in which SAS structure and function are conserved; or
- c) a polypeptide represented by SEQ ID NO: 8; or

d) a variant of the polypeptide represented by SEQ ID NO: 8 that contains conservative amino acid substitutions and in which SAS structure and function are conserved,

said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonoic acid).

227. (Previously presented) The isolated or purified cell of claim 226, which is an insect cell.

228. (Previously presented) The insect cell of claim 227, wherein said insect cell is of a species selected from the group consisting of:

- (a) *Spodoptera frugiperda*;
- (b) *Trichoplusia ni*;
- (c) *Estigmena acrea*; and,
- (d) *Drosophila*.

229. (Previously presented) The isolated or purified cell of claim 226, which is a yeast cell.

230. (Previously presented) The isolated or purified cell of claim 226, which is a plant cell.

231. (Previously presented) The isolated or purified cell of claim 226, which is a bacterial cell.

232. (Previously presented) The isolated or purified cell of claim 226, which is a fungal cell.

233. (Previously presented) The isolated or purified cell of claim 226, which is a mammalian cell.

234. (Previously presented) The isolated or purified cell of claim 226, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.

235. (Previously presented) An isolated or purified cell from a recombinant or genetically engineered cell line which contains and co-expresses

i) a cytidine monophosphate-sialic acid (CMP-SA) synthase gene, wherein said CMP-SA synthase gene encodes a polypeptide that catalyzes the formation of CMP-SA from SA and cytidine triphosphate (CTP), and wherein said CMP-SA synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 3, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:3 under stringent conditions, and

ii) a sialic acid phosphate synthase gene wherein said SAS gene encodes a polypeptide that catalyzes the formation of sialic acid phosphate from N-acetylmannosamine (ManNAc) and phosphoenolpyruvate (PEP), and wherein said sialic acid phosphate synthase gene comprises

a) a nucleotide sequence represented by SEQ ID NO: 5, or

b) a polynucleotide that hybridizes to the nucleotide sequence represented by SEQ ID NO:5 under stringent conditions,

said cell producing a donor substrate CMP-SA at a higher level than a cell from a parent cell line corresponding to said recombinant or genetically engineered cell line, wherein the donor substrate CMP-SA is CMP-KDN (cytidine monophosphate-2-keto-3-deoxy-D-*glycero*-D-*galacto*-nonoic acid).

236. (Previously presented) The isolated or purified cell of claim 235, which is an insect cell.

237. (Previously presented) The insect cell of claim 236, wherein said insect cell is of a species selected from the group consisting of:

(a) *Spodoptera frugiperda*;

(b) *Trichoplusia ni*;

(c) *Estigmena acrea*; and,

(d) *Drosophila*.

238. (Previously presented) The isolated or purified cell of claim 235, which is a yeast cell.

239. (Previously presented) The isolated or purified cell of claim 235, which is a plant cell.

240. (Previously presented) The isolated or purified cell of claim 235, which is a bacterial cell.

241. (Previously presented) The isolated or purified cell of claim 235, which is a fungal cell.

242. (Previously presented) The isolated or purified cell of claim 235, which is a mammalian cell.

243. (Previously presented) The isolated or purified cell of claim 235, wherein said CMP-sialic acid synthase gene and said sialic acid phosphate synthase gene are isolated from a human source.